

Interaction between salt and heat stress: when two wrongs make a right

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In the field, crops are routinely subjected to a combination of different abiotic stresses. Particularly, in arid and semi-arid areas many crops encounter combinations of drought, heat, and salinity that lead to the subsequent overproduction of reactive oxygen species (ROS). Agronomic and physiological studies have revealed that the response of plants to a combination of different abiotic stresses cannot be directly extrapolated from the response of plants to each of the different stresses applied individually (Mittler 2006 and references therein). The same is true at the molecular level: large-scale microarray experiments, in which several abiotic and biotic stresses were studied either in combination or as a single stress, concluded that about 60% of the total transcripts expressed cannot be predicted from the corresponding single stress experiments (Rasmussen *et al.* 2013). These complex interactions may account for: i) the difficulty of extrapolating the results of laboratory research to the field (e.g. tolerance phenotypes of many transgenic plant lines); ii) the function of many still uncharacterized plant genes, ~13% in *Arabidopsis thaliana* (Rasmussen *et al.* 2013); and iii) the unexpectedly high proportion of *A. thaliana* mutant lines exhibiting tolerant or sensitive phenotypes to specific combinations of different abiotic stress treatments (Luhua *et al.* 2012). Therefore, a combination of stresses should be regarded as a new state of abiotic stress in plants that requires new acclimation responses. These findings highlight the importance of studying genetic, biochemical, metabolic and physiological aspects of combined stresses in model and agronomic plant species to facilitate the development of crops with enhanced tolerance to field stress conditions.

In combination, different stresses might require conflicting or antagonistic responses from a plant (Mittler 2006). During heat stress, for example, plants open their stomata to cool their leaves by transpiration. If heat stress is combined with drought, plants may not be able to open their stomata, resulting in a higher leaf temperature than under the heat stress alone (Rizhsky 2002). Salinity might be expected to pose a similar problem for plants when combined with heat stress because enhanced transpiration to

cool the leaf could result in enhanced uptake of toxic ions (Mittler, 2006). However, in this issue of Plant, Cell and Environment, Rivero *et al.* (2013) analyzed primary responses (first 72 hours) to the interaction between salinity and mild heat stress in tomato plants (*Solanum lycopersicum*). The authors show that the combined effect of heat and salt stress, instead of generating the expected additive detrimental consequences on tomato plants performance, induced complementary responses that resulted in a higher degree of resistance compared to salt stress alone. Physiological results pointed to a suite of mechanisms involved in this heat-induced salinity tolerance: i) improved sodium (Na^+) and potassium (K^+) homeostasis, contrary to expectations; ii) improved water balance parameters; iii) higher protection against oxidative stress; iv) a more efficient photosynthetic performance. The comprehensive and multidisciplinary work performed by Rivero *et al.* (2013) explored molecular, biochemical and metabolic responses that could potentially account for the above protecting mechanisms. In particular, the biosynthesis and accumulation of glycine betaine (GB) and trehalose after 48 hours of stress initiation was hypothesized to be a key tolerance factor when heat and salt stresses were imposed simultaneously.

Cross-tolerance is a broadly described phenomenon in plants, whereby a response to one stress also helps to protect the plant from another coincident or subsequent environmental stress. The results by Rivero *et al.* (2013) indicate protection to salt stress following a mild heat stress treatment (35 °C). In plants, acquired thermotolerance is the ability to cope with lethal high temperatures following acclimatization to sublethal high temperatures. It entails physiological adaptations that increase antioxidant activity, chlorophyll stability, and membrane integrity, further reducing ROS accumulation and electrolyte leakage (reviewed in Song et al 2012). These processes are at the core of the tolerance mechanisms described in the work by Rivero *et al.* (2013), indicating that rapid thermotolerance acquisition following the 35 °C treatment is probably a key factor explaining the cross-protection phenomenon observed. This indicates that both environmental stresses induce common tolerance mechanisms in plants and, therefore, the reverse phenomenon should also be expected: thermotolerance acquisition following acclimatization to salt stress. Indeed, Wen *et al.* (2005) reported that acclimation to salt stress induced thermotolerance in the halophyte *Artemisa anethifolia* L. by increasing protection of the photosystem-II (PSII) apparatus. Interestingly, some of the tomato plant responses described by Rivero *et al.* (2013), such as the synthesis of a specific set of osmoprotectant molecules, were unique to the

salt + heat treatment, reinforcing the concept that the plant's response to stress combinations is qualitatively different to the sum of the individual stress responses, and that cross-tolerance is just a part of the story.

The responses derived from the heat + salt treatment and proposed by Rivero *et al.* (2013) as having protective roles over the detrimental consequences of NaCl stress applied alone are summarized in Figure 1. Due to stomatal closure under salt stress the photosynthetic fixation of CO₂ is depressed, and excess of electrons from photosystem-I (PSI) are converted to ROS, which inhibit the repair of photodamaged PSII (Takahashi and Murata 2008). Stomatal opening induced by heat + salt stress allows a higher transpiration rate and facilitates CO₂ diffusion into the leaf, therefore enabling higher CO₂ assimilation rates, higher PSII activity, reduced ROS production, and reduced damage of the photosynthetic apparatus. This explains why the stress combination reduced H₂O₂ accumulation, protein oxidation and proteolytic activity (Rivero *et al.* 2013). Similar results were previously obtained in cashew plants where detrimental effects of salt-stress (NaCl 100 mM) were alleviated by high temperatures (42°C) in terms of a decreased lipid peroxidation and H₂O₂ concentration (Ferreira-Silva *et al.* 2011). Other potentially important mechanism proposed by Rivero *et al.* (2013) to explain improved stress tolerance under the combined stresses was the accumulation of the compatible osmolytes GB and trehalose (Fig. 1). Higher GB production under salt + heat stress can enhance stress tolerance through suppression of ROS accumulation and also via enhanced repair of PSII (Chen & Murata 2011). GB could specifically protect enzymes involved in CO₂ assimilation (Rubisco and Rubisco activase), thereby sustaining the fixation of CO₂, which, in turn, depresses the production of ROS (Takahashi and Murata 2008). Although the protective role of trehalose on the stability of proteins, macromolecules and cellular structures (Crowe 2007) has been questioned due to its low content in plant tissues, the signaling function of the trehalose precursor trehalose-6-phosphate (T6P) on photosynthesis and carbon metabolism (Ponnu *et al.* 2011) may positively regulate the plant's photosynthetic capacity and PSII efficiency. However a direct cause-effect correlation between GB and trehalose (or T6P) accumulation and the higher tolerance for the stresses observed in Rivero *et al.* (2013) must still be established in the context of a specific response to salt + heat combination.

Other results related to the salinity + heat response are more difficult to account for and require further attention (Fig. 1). One of the key findings of this work was the effect of salt + heat treatment on Na⁺/K⁺ homeostasis. The connection between the

combined salt + heat treatment (perhaps through the accumulation of compatible osmolytes) and the long-distance transport of Na^+ and K^+ is not clear. Particularly interesting was the strong improvement of Na^+ exclusion shown by Rivero *et al.* (2013), to the extent that, if confirmed, it may open new strategies for coping with salinity stress in higher plants. Possible mechanisms involved, which deserve further investigation, are heat inactivation of cation transporters involved in root-to-shoot Na^+ translocation at the root xylem parenchyma, and heat-associated induction of mechanisms accounting for the stabilization of cell membranes and/or cation transport mechanisms (e.g. H^+ ATP-ase and Na^+/H^+ antiporters) involved in Na^+ detoxification. Maintaining a higher K^+ content was proposed to be due to a reduction of both K^+ loss from the shoot and K^+ outflow from the root. This, in turn, could be a consequence of a more effective Na^+ exclusion capacity and the proposed protection of plant cell membranes (Cuin and Shabala 2005). A specific role has been proposed for GB in reducing intracellular K^+ efflux caused by ROS either by protecting membrane integrity or through a channel-blocking function (Chen and Murata 2012). Interestingly, despite maintaining a higher rate of transpiration, leaves of plants subjected to heat + salt stress had higher leaf water content and turgor pressure in comparison to the salt treatment alone, apparently due to a more effective osmotic adjustment capacity (Fig. 1; Rivero *et al.* 2013). Since compatible osmolytes have limited osmotic capacity, the protection of cell membranes and a better intracellular retention of key inorganic ions like K^+ , possibly linked to higher GB content (Chen and Murata 2012), may account for the improved water status measured in tomato leaves under salt + heat stress.

However, we must be cautious about generalizing the results until there is a larger body of evidence describing the possible effects of a combination of salt + heat stress in plants. Species- or variety-specific factors, or the intensity and duration of the stress treatments can lead to different results. Çiçek and Çakırlar (2008) found that effects of salt stress and temperature on soybean photosynthetic parameters varied greatly between cultivars and stress intensity. In *Jatropha curcas*, the combination of salt (NaCl 100 mM) with a heat stress treatment of 43 °C (which is stronger than the 35°C used in Rivero *et al.* 2013), was more harmful than any of the stresses applied individually (Silva *et al.* 2012). Although heat stimulated the accumulation of GB in leaves of salt-treated *Jatropha* plants, the combined heat and salt stress strongly impaired stomatal conductance and CO_2 assimilation, and increased Na^+ and Cl^- accumulation in the leaves, which was associated with increased membrane damage and

lipid peroxidation (Silva *et al.* 2012). This result in *Jatropha* indicates that a higher temperature exacerbated the negative effect of salt stress on the same key physiological processes reported by Rivero *et al.* (2013).

In conclusion, it is reasonable to think that plants have evolved specific adaptations to combinations of stresses that are normally associated in nature. But, as described, we should not expect a simple and predictable response pattern. First, the acclimation capacity of a plant subjected to a combination of stresses may vary widely depending on the type, sequence of application, and intensity of the individual stresses implied. Second, besides the interplay involved during signaling and initial molecular responses, further interactions between environmental stresses at the physiological and biochemical levels have proved important in modulating the plant response and acclimation capacity.

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Figure legends

Figure 1. Comparison of early physiological responses of tomato plants to salt stress (120 mM NaCl, 25°C; SALT) or to a combination of salt and mild heat stress (120 mM NaCl, 35°C; SALT + HEAT) according to results obtained by Rivero *et al.* (2013) and others. Primary responses directly modulated by the combination of salt + heat stress are boxed (stomatal opening, and GB and trehalose production). Heat stress induces stomatal opening to facilitate cooling of the leaves, allowing a higher transpiration rate and therefore higher CO₂ assimilation rates, higher PSII activity, reduced ROS production, and reduced damage of the photosynthetic apparatus. Higher GB production under salt + heat stress can enhance tolerance through suppression of ROS accumulation and also via enhanced repair of photosystem-II (Chen & Murata 2011). Apart from the unlikely role of trehalose as an osmoprotectant, trehalose-6-phosphate (T6P) has been proposed to improve plant photosynthetic capacity (Ponnu *et al.* 2012). Other aspects related to the salinity + heat response are less clear and require further study. The higher Na⁺ exclusion and better K⁺ retention capacity under salt + heat stress reduces the Na⁺/K⁺ ratio, which lowers ion toxicity and favours photosynthetic activity. A specific role has been proposed for GB in reducing intracellular K⁺ efflux caused by ROS, either by protecting membrane integrity or through a channel-blocking function (Chen and Murata 2012). This increased retention capacity of K⁺, and possibly other ions in the shoot may be associated in turn with a greater ability to regulate leaf cell

osmolarity, and it may explain the higher water content and turgor pressure measured in tomato leaves under salt + heat stress. All these responses under conditions of salinity + heat may account for the lower Na⁺ toxicity, better water status, higher photosynthetic capacity and greater growth in terms of shoot and root biomass compared with plants subjected to salt stress alone.

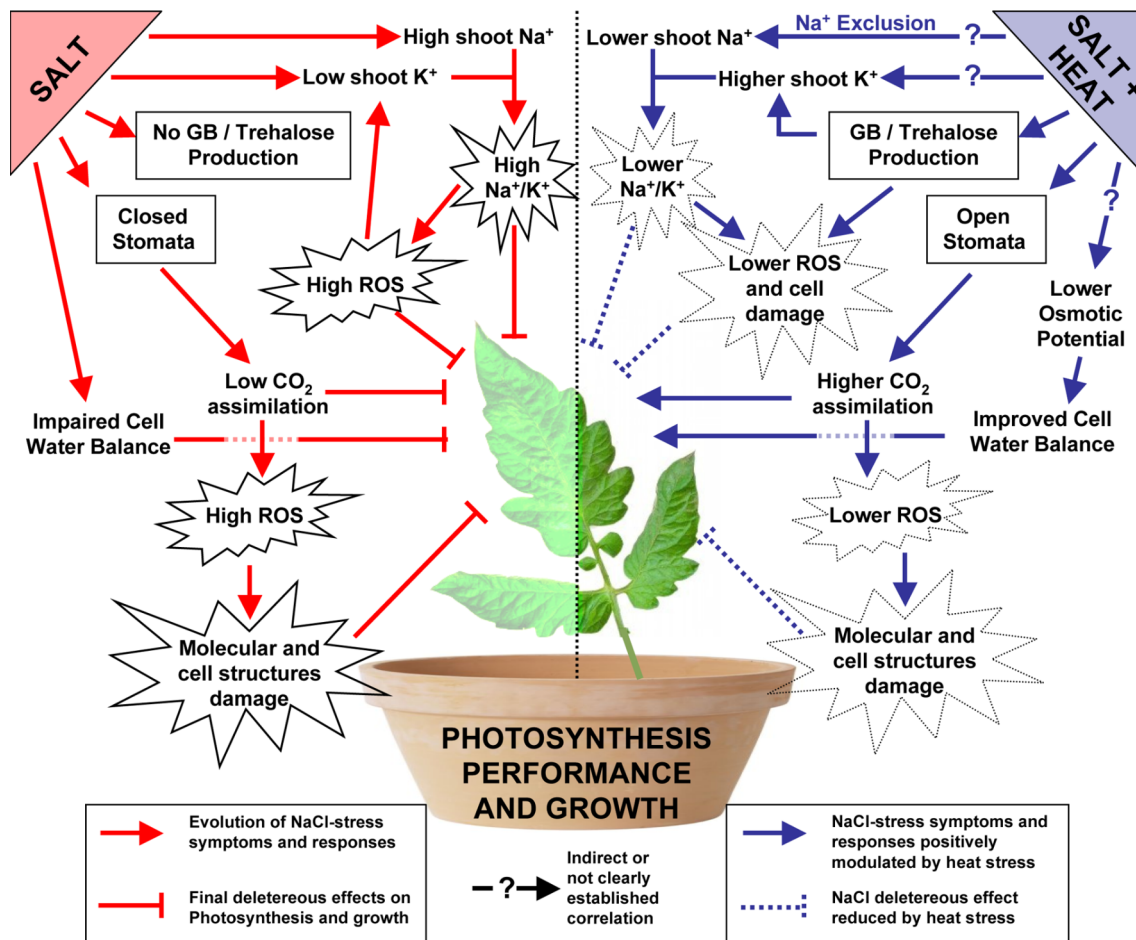


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